Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1419–1423, March 1985
Cell Biology

Interaction of mouse mammary epithelial cells with collagen substrata: Regulation of casein gene expression and secretion

(extracellular matrix/protein degradation/mRNA levels/whey acidic protein)

EVA YUE-HWA P. LEE*†, WEN-HWA LEE‡, CHARLOTTE S. KAETZEL§, GORDON PARRY*, AND MINA J. BISSELL*¶

*Laboratory of Cell Biology, Division of Biology and Medicine, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720; ‡Department of Pathology, University of California at San Diego, La Jolla, CA 92093; and §Department of Pathology, Case Western University, Cleveland, OH 44106

Communicated by Melvin Calvin, October 15, 1984

ABSTRACT **Mouse mammary epithelial cells (MMEC) secrete certain milk proteins only when cultured on floating collagen gels. We demonstrate here that modulation of milk proteins by substrata is manifested at several regulatory levels; (*i*) Cells cultured on floating collagen gels have 3- to 10-fold more casein mRNA than cells cultured on plastic or attached collagen gels. (*ii*) Cells on the latter two "flat" substrata, nevertheless, synthesize a significant amount of caseins, indicating that the remaining mRNA is functional. (*iii*) Cells on all substrata are inducible for casein mRNA and casein proteins by prolactin, but the extent of induction is greater on collagen than that on plastic—i.e., the substratum confers an altered degree of inducibility. (*iv*) Cells on all substrata synthesize casein proteins at rates proportional to the amount of casein mRNA, but the newly synthesized caseins in cells on plastic are degraded intracellularly, whereas those synthesized by cells on floating gels are secreted into the medium. (*v*) Cells on all substrata examined lose virtually all mRNA for whey acidic protein despite the fact that this mRNA is abundant in the mammary gland itself; we conclude that additional, as-yet-unknown, factors are necessary for synthesis and secretion of whey acidic protein in culture.**

Mouse mammary epithelial cells (MMEC) secrete a group of milk-specific proteins, including various caseins and whey proteins (1–3). Dissociated mammary epithelial cells maintain expression of most of their differentiated functions only if cells are plated on a suitable substratum. Collagen extracted from rat tail (4), and matrix prepared from mammary glands of rats in midpregnancy (5, 6), have been used as substrata for culturing mammary epithelial cells (3, 5–10). Casein production and secretion, cell morphology (6–10), and production of α-lactalbumin (5, 6) have been used as markers to assess the degree of differentiation of mammary cells in culture. The consensus is that cells express their differentiated properties at higher levels and for longer periods of time on such substrata (3, 5–10).

Using two-dimensional gel electrophoresis to analyze the secreted proteins of MMEC cultured on tissue culture plastic and attached and floating type I collagen gels, we demonstrated previously that each culture secreted a different spectrum of milk-specific proteins (3). Caseins were secreted mainly by cells cultured on floating collagen gels, whereas transferrin was secreted by cells cultured under all three culture conditions. To understand how the flat substrata impede casein secretion and at what level the floating collagen gel maintains the expression of caseins, we quantified the mRNA for caseins and studied the kinetics of casein synthesis and secretion.

Here we demonstrate that modulation of the expression of caseins by gloating collagen gels is manifested at several regulatory points, including an increase in the levels of mRNA, a decrease in intracellular protein degradation, and an increased ability to secrete the caseins into the medium. In addition, we demonstrate that the floating collagen gel is both permissive and inductive for casein mRNA levels—i.e., the gel allows a preferential induction of casein mRNA in the presence of lactogenic hormones.

MATERIALS AND METHODS

Preparation and Culturing of MMEC. Isolation and culturing of mammary epithelial cells from 14- to 16-day-pregnant BALB/c mice were done as described (3) with the following modifications. After removal of mammary glands, epithelial cells were dissociated by collagenase digestion for 30 min at 37°C. The resulting suspension was spun at 500 rpm for 2 min (IEC HN-5 centrifuge). Top and bottom layers of undissociated clumps were redigested by collagenase. This step was repeated three times. Dissociated cells in the middle layer were collected and recombined for further purification as described (3). Plastic dishes and collagen gel plates were coated with medium F-12 and medium 199 (1:1; GIBCO) containing 10% fetal calf serum for 1 day prior to use. Fetuin (Sigma) at 1 mg/ml was included during plating of the cells. Cell yields and plating efficiency, especially for cultures on plastic dishes, were improved significantly with these modifications.

Collagen was extracted from rat tail tendons and collagen gels were prepared as described previously (3). Gels were released from plastic dishes by circling around the inner edge of the plate with a spatula on day 2 or day 4 after seeding cells. Insulin (5 μg/ml; Sigma), cortisol (2 μg/ml; Sigma), and prolactin (3 μg/ml; National Institutes of Health) were added to culture media unless otherwise indicated.

DNA Quantification. Since there were technical difficulties with measuring total cellular proteins or cell numbers on gels, all experiments were quantified on the basis of total DNA. DNA was measured by addition of Hoechst dye to cell lysates followed by fluorometric measurements as described (11). Salmon sperm DNA was used as a standard.

Preparation of Antisera Against Mouse Milk Proteins. Caseins were isolated from skim milk by repeated acid precipitation (pH 4.6), and residual albumin was removed by Affi-Gel Blue (Bio-Rad) chromatography. Acid precipitation yielded a fraction that contained various caseins and transferrin but very little albumin as analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. The proteins were mixed with Freund's complete and incomplete adjuvant

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MMEC, mouse mammary epithelial cells; WAP, whey acidic protein.

†Present address: See ‡.
¶To whom reprint requests should be addressed.

(Difco) and injected subcutaneously into rabbits in multiple sites. Subcutaneous boosts were repeated twice at 4-week intervals. High-titer antibodies against transferrin, albumin, and various caseins were present in this antiserum.

Labeling and Immunoprecipitation Experiments. Cells were incubated in methionine-free medium for 1 hr followed by a 3-hr incubation in the same medium supplemented with [^{35}S]methionine at 100 μCi/ml (1400 Ci/mmol, Amersham; 1 Ci = 37 GBq). For chase experiments, unlabeled methionine was added to the medium at 4 mg/ml to stop the incorporation of radiolabel into proteins. Kallikrein inactivator (200 units/ml, Calbiochem), a proteinase inhibitor, was added to the collected media after pulse or pulse–chase experiments. Cell extracts were prepared as described (3), and aliquots of cellular lysate or media representing equal amounts of DNA were used for immunoprecipitation (3). The immunoprecipitated proteins were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis as described (12).

RNA Dot-Blotting Analysis. This was performed essentially as described (13, 14). After extraction and precipitation with ethanol, RNA was quantified and dotted directly onto a nitrocellulose membrane. Filters were baked at 80°C for 2 hr under reduced pressure, incubated for 24 hr in 50% (vol/vol) formamide/0.75 M NaCl/0.075 M sodium citrate/0.2% bovine serum albumin/0.2% Ficoll/0.2% polyvinyl pyrrolidone and then hybridized with ^{32}P-labeled, nick-translated mouse caseins or whey acidic protein (WAP) cDNA probes (15). Washing was done in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$ at 55°C followed by autoradiography of the dried filters for the necessary time period. The specificities of probes used in these experiments were confirmed with DNA ("northern") blotting (16).

RESULTS

Time Course of Milk Protein Expression. Mammary epithelial cells freshly isolated from mice in midpregnancy or cells cultured on plastic dishes, attached collagen gels, and floating collagen gels were labeled with [^{35}S]methionine for 3 hr at indicated times after plating. Samples were prepared and run on $NaDodSO_4$/polyacrylamide gel electrophoresis as described in *Materials and Methods*. Freshly isolated MMEC actively synthesized and secreted transferrin, and α-, β-, γ-, and δ-casein (Fig. 1, day 0; nomenclature as in ref. 17). After culturing on plastic dishes for 3 days, cells continued to synthesize a significant amount of transferrin and β-casein and a small amount of α1-casein. However, while transferrin was actively secreted into the medium, β-casein remained intracellular (Fig. 1, lanes A). After 12 days in culture, cells on plastic started to detach from the dish and the synthesis and secretion of all milk proteins were further reduced. In contrast, cells on floating gels maintained active synthesis and secretion of caseins and transferrin throughout the entire culture period (Fig. 1, lanes C), which was as long as 1 month (the longest period examined; data not shown). Cells plated on attached collagen gels were similar to cells on floating gels for the first 3 days, but from then on they behaved essentially as cells on plastic (Fig. 1, lanes B).

Quantitative Comparison of Casein mRNA in Cultures on Different Substrata. Total cytoplasmic RNA was extracted from cells cultured for 6 days in the presence of insulin, hydrocortisone, and prolactin and from 6-day lactating mammary gland. RNA was dotted onto nitrocellulose paper and hybridized with known casein probes (15). It was found that the β-casein mRNA levels in cells cultured on floating gels approached those in the lactating gland itself (Fig. 2). However, cells on plastic and attached collagen gels exhibited mRNA levels that were lower by a factor of 3–10. Similar

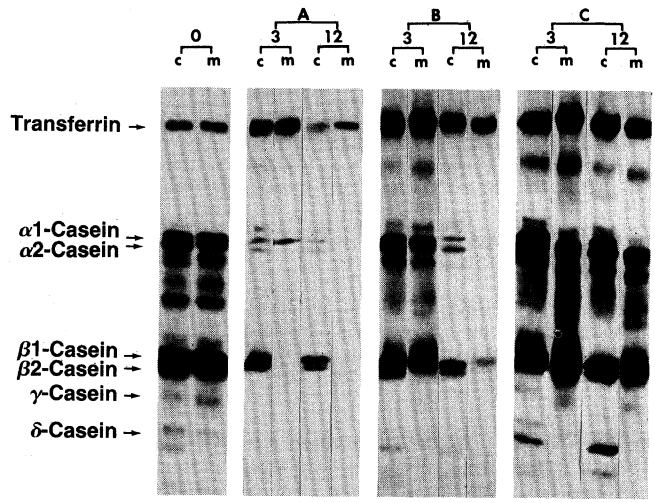

FIG. 1. Time course of production and secretion of milk proteins on plastic and on attached and floating gels. Mammary epithelial cells from 14- to 16-day-pregnant mice were labeled immediately after isolation (0) or cultured on different substrata and labeled on day 3 or 12 as indicated. Samples were immunoprecipitated and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis. Fluorographs were obtained after 3-day exposure to x-ray film. Lanes: A, plastic; B, attached gels; C, floating gels; c, cellular proteins; m, media proteins.

results were obtained for α1-casein mRNA (data not shown). In contrast, WAP mRNA was abundant in the lactating gland but was present only in very small quantities in cells on floating collagen gels, and it was totally undetectable in cultures on plastic and attached gels.

Response to Lactogenic Hormones. To test the possibility that loss of ability to synthesize and secrete caseins correlates with inability to respond to induction by lactogenic hormones, cells were cultured in media supplemented with insulin and hydrocortisone for 5 days. Prolactin was then added for 36 hr. Cells were labeled for the last 24 hr with [^{35}S]methionine. Control cultures did not receive any prolactin. Transferrin was produced and secreted regardless of whether or not prolactin was present, and α1-casein was also expressed at low levels (Fig. 3). However, upon addition of prolactin, α2-casein and β-casein were induced dramatically in cells on collagen gels and appreciably even in cells on plastic (Fig. 3). This observation was further confirmed by determination of casein mRNA by using RNA dot-blot analysis. After prolactin addition, β-casein mRNA increased roughly 10-fold in cells on plastic, 20-fold in cells on attached gels, and 50-fold in cells on floating gels (Fig. 4). This was not a reflection of a general increase in transcription rate since an internal control, that of mRNA of heat shock

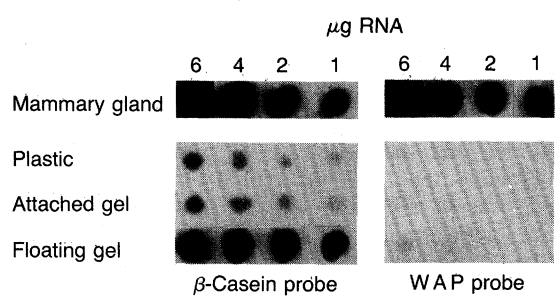

FIG. 2. Quantification of β-casein mRNA and WAP mRNA. RNA was extracted and processed from 6-day cultures or 8-day lactating glands with phenol/chloroform, dotted onto nitrocellulose, and hybridized with probes.

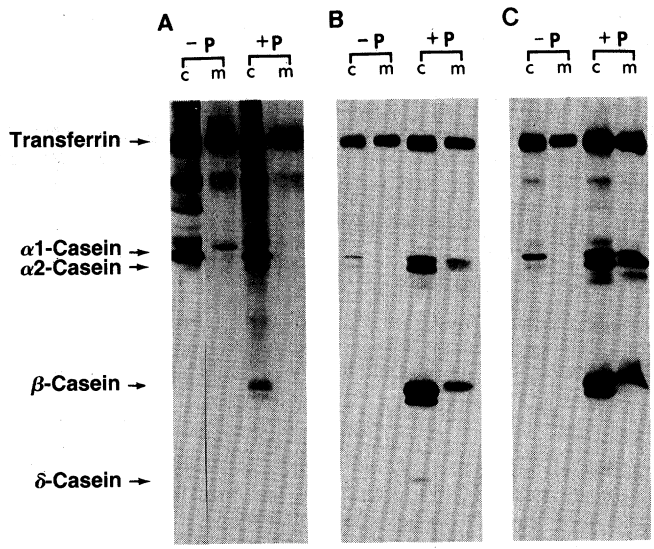

FIG. 3. Induction of milk proteins by prolactin. Cells were cultured in medium containing hydrocortisone and insulin for 5 days. Prolactin was added to one half of the cultures on day 6 (+P). Other details are as in the legend of Fig. 1.

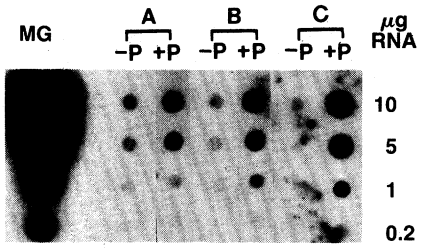

FIG. 4. Induction of casein mRNA in MMEC by prolactin. Cells were cultured as described for Fig. 3 and RNA was extracted and dotted as described in *Materials and Methods*. MG, lactating mammary gland RNA.

protein hybridized to its cDNA clone, was essentially unchanged upon prolactin addition (data not shown).

Heterogeneity of Casein-Producing Cells on Plastic. Mammary cells on plastic retain some of their epithelial characteristics, including the ability to form domes (Fig. 5 *Upper*). The low level of casein production in these cells is either because a few cells express the expected levels of casein or all cells express a very low level of casein. To distinguish between these two possibilities, indirect immunostaining with monoclonal antibody to β-casein (18) was performed. We found that fewer than 10% of the cells were casein-positive. While casein-synthesizing cells were distributed in both dome and nondome areas (Fig. 5 *Lower*), the areas around the dome showed much stronger fluorescence. Thus the reduced level of casein synthesis in cells on plastic appears to be related to the topology of the monolayer. Haeuptle *et al.* (9) have shown that 30% of mammary epithelial cells from pregnant rabbits are positive for caseins when embedded in collagen gels. Our protein and mRNA data with cells on the floating gels suggest higher numbers than those found for cells on plastic or in embedded gels.

Kinetic Studies of Synthesis and Secretion of Caseins. Despite the fact that we observed 1/3rd to 1/10th as much total casein mRNA in cells on plastic as in those on floating gels, there was no obvious reason why caseins were not detected in the medium of cultures on plastic, since intracellular levels of caseins were easily detectable. To understand this phenomenon, we studied the kinetics of casein synthesis and secretion by pulse-chase labeling with [^{35}S]methionine

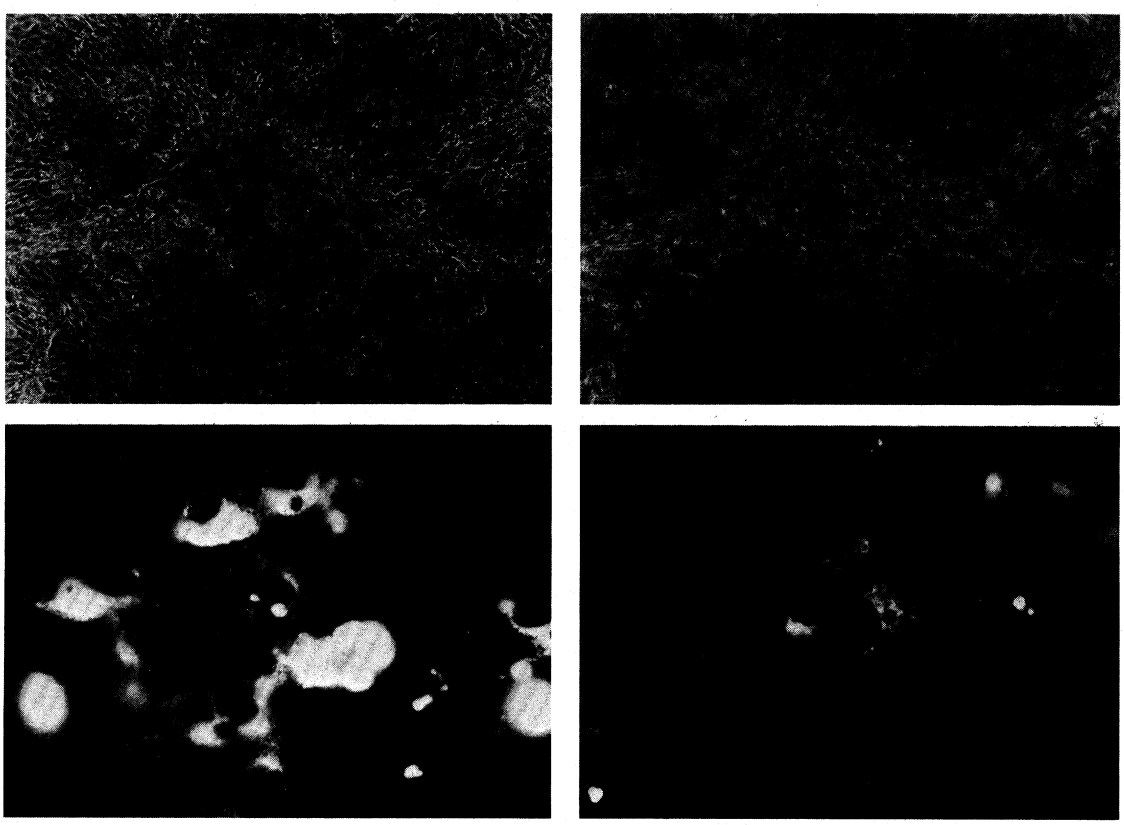

FIG. 5. Dome formation and immunostaining with monoclonal antibody to β-casein in cells cultured on coverslips. Phase-contrast micrographs (*Upper*) show different two planes of the same field to indicate the depth of the dome formed. (×40.) Immunofluorescence micrographs (*Lower*) show β-casein-positive cells in the dome (*Left*) and nondome (*Right*) regions. (×800.)

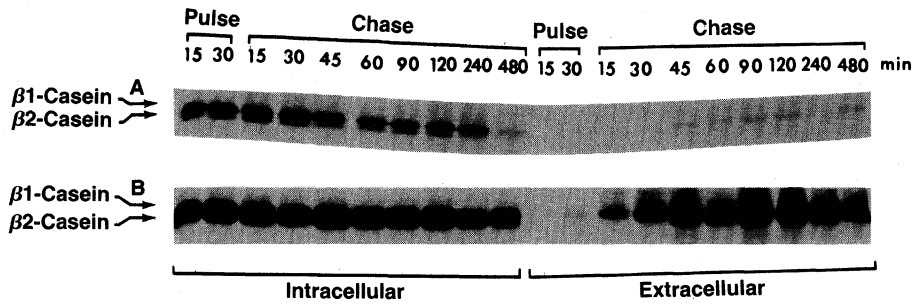

FIG. 6. Pulse–chase labeling of β-casein. Cells on plastic (*A*) or on floating gels (*B*) were incubated in methionine-free medium for 1 hr and then pulsed with [^{35}S]methionine at 150 μCi/ml. One dish was harvested after a 15-min pulse; the rest were pulsed for 30 min, then washed once with medium containing excess unlabeled methionine and chased for various periods. Some extracellular degradation of caseins occurs in the media of cells on both plastic dishes and collagen gels.

as shown in Figs. 6 and 7. Maximal incorporation of [^{35}S]methionine into caseins was seen at 30 min. There was a 5-fold difference in the rate of casein synthesis between cells on plastic and floating gels, consistent with the previous finding of a 3- to 10-fold difference in total casein mRNA (Fig. 2). These results suggest that the translatabilities of casein mRNAs in cells on plastic and collagen are similar. After an 8-hr chase period, 90% of the newly synthesized casein in cells on plastic was degraded intracellularly and 10% was secreted into the medium (Fig. 6*A*). In contrast, the reduction in intracellular levels of caseins (50%) in cells on floating gels was accounted for by secretion into the medium. Intracellular caseins, however, could not be chased out completely from cells on floating gels even after 8 hr. The possibility that the specific activity of the amino acid pool had not been sufficiently reduced to effectively terminate the incorporation of label into proteins was excluded by the fact that, in the same experiments, transferrin was chased out effectively (unpublished data). Furthermore, since secretion by transferrin is unaffected by the nature of the substrata (Fig. 1), these results demonstrate a specific impairment in casein secretion for cells on plastic.

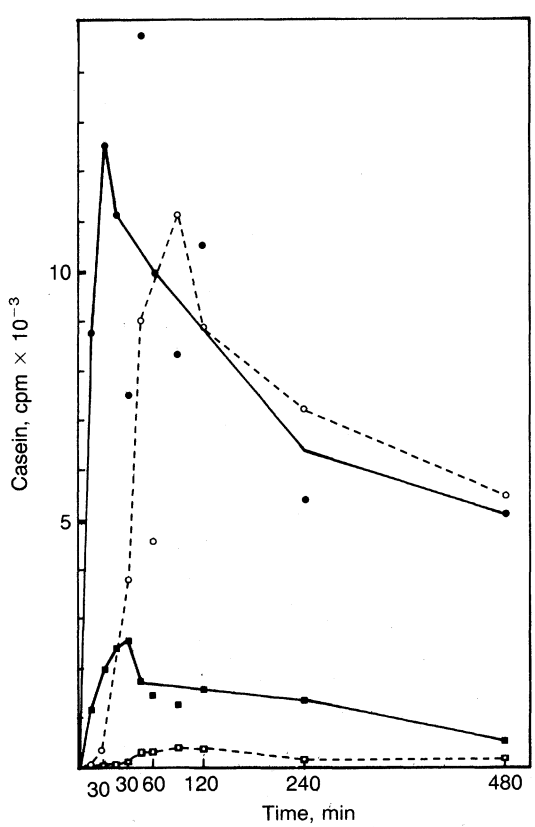

FIG. 7. Kinetics of β-casein synthesis and secretion. Radiolabeled β-casein in bands shown in Fig. 6 was excised and its radioactivity was measured in a scintillation counter and plotted. ——, Intracellular casein; – – –, extracellular casein; ○, floating gels; □, plastic dishes. The numbers on the abscissa refer to 30 min of pulse, followed by the indicated chases.

DISCUSSION

Collagen substrata appear to influence the expression of milk proteins at several regulatory levels. From the time-course studies it is apparent that cells on plastic initially synthesize significant amounts of caseins, but there is an impairment of casein secretion. The decrease in casein synthesis that occurs in mature cultures is thus a later event. It is also clear that the substrata regulate the rate of synthesis and/or processing of casein mRNA. A 3-fold higher level of casein mRNA in floating gel cultures was reported earlier by Supowit *et al.* (19), but prolactin induction was very limited and protein data were not included. In contrast to Supowit *et al.*, and our findings, Suard *et al.* (10) reported a virtual absence of casein mRNA and casein proteins in 8-day attached gel cultures of rabbit mammary epithelial cells. While these discrepancies may be due to species differences, they are more likely due to differences in culture conditions or lower efficiency of hybridization and immunoprecipitation in previous studies.

The pioneering work of Emerman *et al.* (7, 8), while demonstrating higher levels of synthesis and secretion of γ-casein by cells on floating gels than those on plastic or attached gels, appeared to indicate that such differences may be due to an inability to respond to prolactin. However, some modulation of milk protein synthesis by lactogenic hormones has been demonstrated for cells on plastic (20, 21). Using a γ-casein probe, we have observed higher levels of γ-casein mRNA in all cultures upon prolactin addition (data not shown); this was accompanied by increased levels of the protein. While mouse milk contains a significant amount of γ-casein (3, 16), its level of expression in culture is low as compared to α- or β-caseins (ref. 3 and this paper). Thus it is possible that immunoassay used in previous studies might not have been sensitive enough to detect the γ-casein induction in culture. Our results indicate that cells on all substrata retain the capability of responding to prolactin, but the magnitude of induction of both mRNA and protein is smaller on plastic and attached gels than on floating gels. It is also likely that only cells cultured on floating gels are capable of stabilizing and accumulating casein mRNA in long-term exposure to prolactin. It should be pointed out that induction of various caseins in culture is not coordinate; e.g., α1-casein was expressed at low levels whether or not

prolactin was present, while α2- and β-casein were induced most dramatically upon prolactin addition. Furthermore, the response to hormones may be different in different species. It was shown in rat mammary organ cultures that prolactin addition caused a marked increase in γ-casein while α- and β-casein were less affected. Regulation of WAP appears to be even more uncoordinate in that in organ culture it is mainly regulated by hydrocortisone but not prolactin (22). Our results indicate that in cell culture WAP is regulated neither by lactogenic hormones nor by substratum, and the same appears to be true for α-lactalbumin. This culture system, therefore, could be used to decipher the additional factors that may be responsible for WAP and α-lactalbumin expression *in vivo*.

Finally, collagen substrata affect the differentiation status of MMEC also at the level of casein degradation. Our data indicate a significant decrease in intracellular casein degradation and a great enhancement of casein secretion when cells are grown on floating collagen gels. Extensive degradation of caseins has been reported in mammary organ cultures (23), where 90% of the synthesized caseins remained inside the cells, indicating that degradation in this case may also be linked to the failure to secrete. Intracellular and extracellular degradation of phosphocaseins in freshly dissociated mammary cells has been reported recently (6), although delayed incorporation of radiolabels into phosphoproteins in these studies may indicate an impairment of protein synthesis in such suspended cultures. Whether or not the extensive intracellular degradation reported in these (6, 23) and our studies occurs *in vivo* awaits further investigation.

The impairment of secretion by cells on plastic may be due to changes in posttranslational modification of caseins. Our preliminary data suggest that caseins in cells on plastic are phosphorylated to a lesser degree than those on floating gels and that secreted caseins are highly phosphorylated when measured by double labeling with [^{35}S]methionine and [^{32}P]phosphate (unpublished data).

How does the extracellular matrix or its components affect gene expression, posttranslational modification, and secretion? It was shown in thyroid cells cultured on attached gels that the lack of response to acute stimulation by thyroid-stimulating hormone (TSH) and failure to concentrate iodide was due to the inaccessibility of iodide and TSH to the basolaterally localized iodide pump and the TSH receptor–adenylate cyclase complex (24). In the mammary epithelial system, the accessibility of hormones and other components of the medium to the basolateral surface is not the main factor, since mammary epithelial cells cultured on floating glutaraldehyde-treated collagen gels still failed to secrete caseins (3, 25). The change in cell shape brought about by floating gels (7, 9) may be important in casein synthesis and secretion, and in this context it is interesting to note that cells within the dome areas on plastic are the ones synthesizing the highest level of caseins. Support for a relationship between cell shape changes and expression of differentiated functions has come not only from studies in the mammary gland (7, 9) but also from other systems (26, 27). Bissell *et al.* (28) have proposed a network of interacting components composed of the extracellular matrix, transmembrane receptors, cytoskeleton, and nuclear matrix, all contributing to the maintenance of the differentiated state. The ultimate mechanisms relating the cell shape, the cytoskeleton, and the components of the extracellular matrix of mammary cells (29) to that of casein gene expression merit further investigation.

We thank Dr. Jeffrey M. Rosen for the mouse milk protein cDNA clones, Debbie Farson for editorial comments, and Joan Graham for typing the manuscript. This work was supported by the Office of Health and Environmental Research, Office of Energy Research, U.S. Department of Energy, Contract no. DE-AC03-76SF00098.

1. Green, M. R. & Pastwka, J. V. (1976) *J. Dairy Sci.* **59,** 207–215.
2. Piletz, J. E., Heinlen, M. & Ganshow, R. E. (1981) *J. Biol. Chem.* **256,** 11509–11516.
3. Lee, E. Y.-H., Parry, G. & Bissell, M. J. (1984) *J. Cell Biol.* **98,** 146–155.
4. Michalopoulos, G. & Pitot, H. C. (1975) *Exp. Cell Res.* **94,** 70–78.
5. Wicha, M. S., Lowrie, G., Kohn, E., Bagavandoss, P. & Mahn, T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3213–3217.
6. Wilde, C. J., Hasan, H. R. & Mayer, R. J. (1984) *Exp. Cell Res.* **151,** 519–532.
7. Emerman, J. T. & Pitelka, D. R. (1977) *In Vitro* **13,** 316–327.
8. Emerman, J. T., Enami, J., Pitelka, D. R. & Nandi, S. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4466–4470.
9. Haeuptle, M.-T., Suard, Y. L. M., Bogenmann, E., Reggio, H., Racine, L. & Kraehenbuhl, J.-P. (1983) *J. Cell Biol.* **96,** 1425–1434.
10. Suard, Y. M. L., Haeuptle, M.-T., Farinon, E. & Kraehenbuhl, J.-P. (1983) *J. Cell Biol.* **96,** 1435–1442.
11. Labarca, C. & Paigen, K. (1980) *Anal. Biochem.* **102,** 344–352.
12. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
13. White, B. & Bancroft, F. (1982) *J. Biol. Chem.* **257,** 8569–8572.
14. Lee, W.-H., Murphree, A. L. & Benedict, W. F. (1984) *Nature (London)* **309,** 458–460.
15. Gupa, P., Rosen, J. M., D'Eustachio, P. & Ruddle, F. H. (1982) *J. Cell Biol.* **93,** 199–204.
16. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5350–5353.
17. Hennighausen, L. G. & Sippel, A. E. (1982) *Eur. J. Biochem.* **125,** 131–141.
18. Kaetzel, C. S. & Durwood, R. (1984) *J. Dairy Sci.* **67,** 64–75.
19. Supowit, S. C., Asch, B. B. & Rosen, J. M. (1981) in *Cell Biology of Breast Cancer*, eds. McGrath, C., Brennon, M. & Rich, M. (Academic, New York), pp. 247–263.
20. Ray, D. B., Horst, I. A., Jansen, R. W. & Kowal, J. (1981) *Endocrinology* **108,** 573–583.
21. Ray, D. B., Horst, I. A., Mills, N. C. & Kowal, J. (1981) *Endocrinology* **108,** 584–590.
22. Hobbs, A. A., Richards, D. A., Kessler, D. J. & Rosen, J. M. (1982) *J. Biol. Chem.* **257,** 3598–3605.
23. Hasan, H. R., White, D. A. & Mayer, R. J. (1982) *Biochem. J.* **202,** 133–138.
24. Chambard, M., Gabrion, V. J. & Mauchamp, J. (1983) *J. Cell Biol.* **96,** 1172–1177.
25. Shannon, J. M. & Pitelka, D. R. (1981) *In Vitro* **17,** 1016–1028.
26. Benya, P. D. & Shaffer, J. D. (1982) *Cell* **30,** 215–224.
27. Ingber, D. E. & Jamieson, J. D. (1984) in *Gene Expression During Normal and Malignant Differentiation*, eds. Andersson, L. C., Gahmberg, C. G. & Ekblou, P. (Academic, New York), in press.
28. Bissell, M. J., Hall, G. H. & Parry, G. (1982) *J. Theor. Biol.* **99,** 31–68.
29. Parry, G., Lee, E. Y.-H., Farson, D., Koval, M. & Bissell, M. J. (1984) *Exp. Cell Res.*, in press.